

SEQUENCE LISTING

<110> Brookhaven Science Associates
Shanklin, John

<120> Mutant Fatty Acid Desaturase and Methods for Directed Mutagenesis

<130> CIP of 09/328,550 filed June 9, 1999; which was a CIP of 09/233,856
filed January 19, 1999

<150> 09/328,550
<151> 1999-06-09

<160> 13

<170> PatentIn version 3.1

<210> 1
<211> 363
<212> PRT
<213> Ricinus communis

<220>
<221> misc_feature
<223> ricinus communis delta 9 18:0 Acyl ACP Desaturase

<400> 1

Ala Ser Thr Leu Lys Ser Gly Ser Lys Glu Val Glu Asn Leu Lys Lys
1 5 10 15

Pro Phe Met Pro Pro Arg Glu Val His Val Gln Val Thr His Ser Met
20 25 30

Pro Pro Gln Lys Ile Glu Ile Phe Lys Ser Leu Asp Asn Trp Ala Glu
35 40 45

Glu Asn Ile Leu Val His Leu Lys Pro Val Glu Lys Cys Trp Gln Pro
50 55 60

Gln Asp Phe Leu Pro Asp Pro Ala Ser Asp Gly Phe Asp Glu Gln Val
65 70 75 80

Arg Glu Leu Arg Glu Arg Ala Lys Glu Ile Pro Asp Asp Tyr Phe Val
85 90 95

Val Leu Val Gly Asp Met Ile Thr Glu Glu Ala Leu Pro Thr Tyr Gln
100 105 110

Thr Met Leu Asn Thr Leu Asp Gly Val Arg Asp Glu Thr Gly Ala Ser
 115 120 125

Pro Thr Ser Trp Ala Ile Trp Thr Arg Ala Trp Thr Ala Glu Glu Asn
 130 135 140

Arg His Gly Asp Leu Leu Asn Lys Tyr Leu Tyr Leu Ser Gly Arg Val
 145 150 155 160

Asp Met Arg Gln Ile Glu Lys Thr Ile Gln Tyr Leu Ile Gly Ser Gly
 165 170 175

Met Asp Pro Arg Thr Glu Asn Ser Pro Tyr Leu Gly Phe Ile Tyr Thr
 180 185 190

Ser Phe Gln Glu Arg Ala Thr Phe Ile Ser His Gly Asn Thr Ala Arg
 195 200 205

Gln Ala Lys Glu His Gly Asp Ile Lys Leu Ala Gln Ile Cys Gly Thr
 210 215 220

Ile Ala Ala Asp Glu Lys Arg His Glu Thr Ala Tyr Thr Lys Ile Val
 225 230 235 240

Glu Lys Leu Phe Glu Ile Asp Pro Asp Gly Thr Val Leu Ala Phe Ala
 245 250 255

Asp Met Met Arg Lys Lys Ile Ser Met Pro Ala His Leu Met Tyr Asp
 260 265 270

Gly Arg Asp Asp Asn Leu Phe Asp His Phe Ser Ala Val Ala Gln Arg
 275 280 285

Leu Gly Val Tyr Thr Ala Lys Asp Tyr Ala Asp Ile Leu Glu Phe Leu
 290 295 300

Val Gly Arg Trp Lys Val Asp Lys Leu Thr Gly Leu Ser Ala Glu Gly
 305 310 315 320

Gln Lys Ala Gln Asp Tyr Val Cys Arg Leu Pro Pro Arg Ile Arg Arg
 325 330 335

Leu Glu Glu Arg Ala Gln Gly Arg Ala Lys Glu Ala Pro Thr Met Pro

340 345 350

Phe Ser Trp Ile Phe Asp Arg Gln Val Lys Leu
 355 360

<210> 2

<211> 1092

<212> DNA

<213> Ricinus communis

<220>

<221> misc feature

<223> residues 138 to 1239 of open reading frame

<400> 2

| | |
|---|------|
| gcctctaccc tcaagtctgg ttctaaggaa gttgagaatc tcaagaagcc tttcatgcct | 60 |
| cctcgggagg tacatgttca gtttacccat tctatgccac cccaaaagat tgagatcttt | 120 |
| aaatccctag acaattgggc tgaggagaac attctggttc atctgaagcc agttgagaaa | 180 |
| tgttggcaac cgccaggattt tttgccagat cccgcctctg atggatttga tgagcaagtc | 240 |
| agggaaactca gggagagagc aaaggagatt cctgatgatt attttgttgt tttggttgga | 300 |
| gacatgataa cggaagaagc cttcccact tatcaaacaa tgctgaatac cttggatgga | 360 |
| gttcgggatg aaacaggtgc aagtcctact tcttggcaa tttggacaag ggcattggact | 420 |
| gcggaaagaga atagacatgg tgaccccttc aataagtatc tctacctatc tggacgagtg | 480 |
| gacatgagggc aaattgagaa gacaattcaa tatttggattt gttcaggaat ggatccacgg | 540 |
| acagaaaaaca gtccataacct tgggttcatc tatacatcat tccaggaaag ggcaaccctc | 600 |
| atttctcatg ggaacactgc ccgacaagcc aaagagcatg gagacataaa gttggctcaa | 660 |
| atatgtggta caattgctgc agatgagaag cgccatgaga cagcctacac aaagatagtg | 720 |
| gaaaaactct ttgagattga tcctgtatgg actgtttgg cttttgtatc tatgtatgaga | 780 |
| aagaaaattt ctatgcctgc acacttgatg tatgtatggcc gagatgataa tcttttgac | 840 |
| cactttcag ctgttgcgca gcgtcttggc gtctacacag caaaggatta tgcagatata | 900 |
| ttggagttct tgggtggcag atgaaagggtg gataaactaa cgggccttc agctgaggga | 960 |
| caaaaggctc aggactatgt ttgtcggtt cctccaagaa tttagaaggct ggaagagaga | 1020 |
| gctcaaggaa gggcaaagga agcacccacc atgcctttca gctggatttt cgataggcaa | 1080 |
| gtgaagctgt ag | 1092 |

```

<210> 3
<211> 34
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<223> PCR primer; sequence flanking unique XbaI site at the 5' end of t
he open reading frame

<400> 3
gtgagcggat aacaatttca cacagtctag aaat 34

<210> 4
<211> 72
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<222> (56)..(57)
<223> PCR primer is a degenerate oligonucleotide in which "n" indicates
the presence of either C, A, T or G at that nucleotide position

<400> 4
ccaaattgcc caagacgtcg gacttgcacc tgtttcatcc cgaactccat ccaamnnatt 60
cagcattgtt tg 72

<210> 5
<211> 31
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<223> PCR primer

<400> 5
gaaacaggtg caagtccgac gtcttggca a 31

<210> 6
<211> 26
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<223> PCR primer

```

| | |
|---|----|
| <400> 6 | |
| gttttctgtc cgccggatcca ttcctg | 26 |
| | |
| <210> 7 | |
| <211> 34 | |
| <212> DNA | |
| <213> Artificial | |
| | |
| <220> | |
| <221> misc_feature | |
| <223> PCR primer | |
| | |
| <400> 7 | |
| gtgagcggat aacaatttca cacagtctag aaat | 34 |
| | |
| <210> 8 | |
| <211> 30 | |
| <212> DNA | |
| <213> Artificial | |
| | |
| <220> | |
| <221> misc_feature | |
| <223> PCR primer | |
| | |
| <400> 8 | |
| cacgaggccc tttcgtcttc aagaattctc | 30 |
| | |
| <210> 9 | |
| <211> 28 | |
| <212> DNA | |
| <213> Artificial | |
| | |
| <220> | |
| <221> misc_feature | |
| <223> PCR primer | |
| | |
| <400> 9 | |
| ttgataagtg ggaaggcctt cttccgtt | 28 |
| | |
| <210> 10 | |
| <211> 66 | |
| <212> DNA | |
| <213> Artificial | |
| | |
| <220> | |
| <221> misc_feature | |
| <222> (41)..(43) | |
| <223> PCR primer is a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G and in which "k" indicates the presence of either T or G. | |

```

<220>
<221> misc_feature
<222> (32)..(34)
<223> PCR primer is degenerate oligonucleotide in which "n" indicates th
e presence of either C, A T, or G at that nucleotide position and
in which "k" indicates either T or G

<220>
<221> misc_feature
<222> (44)..(46)
<223> PCR primer is a degenerate oligonucleotide in which "n" indicates
the presence of either C, A, T, or G at that nucleotide position
and in which "k" indicates the presence of either T or G.

<400> 10
aacggaagaa gcccttccca cttatcaaac annkctgaat nnknnkgatg gagttcggga      60
tgaaac                                         66

<210> 11
<211> 26
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<223> PCR primer

<400> 11
tccatttcctg aaccaatcaa atattg                                         26

<210> 12
<211> 70
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<222> (22)..(24)
<223> PCR primer in a degenerate oligonucleotide in which "n" indicates
the presence of either C, A, T or G at that nucleotide position
and in which "k" indicates the presence of either T or G at that
nucleotide position.

<220>
<221> misc_feature
<222> (28)..(30)
<223> PCR primer in a degenerate oligonucleotide in which "n" indicates
the presence of either C, A, T or G at that nucleotide position

```

and in which "k" indicates the presence of either T or G at that nucleotide position.

<220>
<221> misc_feature
<222> (49)..(51)
<223> PCR primer in a degenerate oligonucleotide in which "n" indicates the presence of either C, A, T or G at that nucleotide position and in which "k" indicates the presence of either T or G at that nucleotide position.

<400> 12
ttgattgggtt caggaatgga tnnkcggnnk gaaaacagtc catacctnn kttcatctat 60
acatcattcc 70

<210> 13
<211> 30
<212> DNA
<213> Artificial

<220>
<221> misc_feature
<223> PCR primer

<400> 13
gcaaaagcca aaacggtacc atcaggatca 30